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Potential Dissipation of Atrazine in the Soil Unsaturated Zone: a Comparative Study in Four European Countries*

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Abstract: A European-wide project has been undertaken to establish the potential for dissipation of atrazine in the soil subsurface environment. Samples were obtained, avoiding contamination, in four countries (Belgium, Greece, Hungary and UK) and laboratory studies carried out. In order to make comparisons between results from each laboratory, a ring experiment was carried out using common methodology for sampling, extraction and analytical techniques.

Subsurface materials from each country were distributed to the other countries. Atrazine dissipation was determined in each country for all materials under the same laboratory conditions. The results of this comparative study showed generally good agreement between all laboratories. Significant potential microbiological dissipation was detected in certain samples. Where differences occurred between laboratories this was attributed to small, spatially heterogeneous microbial populations in the subsurface materials.

Key words: atrazine, dissipation, subsurface, methodology, ring test

1 INTRODUCTION

The detection of pesticides in drinking water has stimulated interest in the fate of these compounds in the subsurface environment.[1] Atrazine has been detected in groundwater in many agricultural areas around the world[2–4] but little is known of the potential for dissipation of atrazine in the unsaturated zone. Pesticides such as atrazine may be susceptible to microbial degradation in the subsurface environment but at a slower rate than in surface soil.[5–11]

We have carried out a collaborative study on the potential dissipation of atrazine in samples taken from the unsaturated zone in the UK, Belgium, Greece and Hungary. The technical difficulties involved in such a project concerned with microbiological processes fall into three main areas: (a) obtaining uncontaminated samples from several metres depth (b) establishing a

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common protocol and (c) ensuring repeatability of results between four laboratories. The first point will be addressed elsewhere. The second and third points will be considered in this paper which is a report of a ring test carried out in each of the participating laboratories.

The objective of this study was to use a common protocol in all laboratories using the same materials to measure (a) total viable bacteria and (b) rate of dissipation of atrazine.

2 MATERIALS AND METHODS

Atrazine dissipation was determined in each participating laboratory for all materials using an agreed methodology for incubation, sampling, extraction and analytical techniques. Table 1 shows the locations and the depths of the samples used in this study.

2.1 Obtaining samples

A 200-mm diameter core-barrel fitted with a 50- to 80-cm-long plastic liner was pushed into the ground using a percussion drilling apparatus to obtain core materials from different depths.[12] This was carried out by the Institute of Hydrology, Wallingford, UK for all countries except Hungary where local equipment was used. Subsamples of the core material were removed aseptically from the centre of the core, placed in sterilised containers and well mixed to create homogeneous subsamples. The samples were transported between countries using cool containers.

2.2 Treatments

Atrazine (99% purity) stock solution of 500 mg litre^{-1} was prepared in methanol and analytical standards were prepared by serial dilution with acetonitrile. Atrazine solution of known concentration was also prepared by serial dilution with sterilised deionised water. A known amount of this solution was added to the subsurface materials (200 g, oven-dry basis) in sterilised glass containers (500 ml) to give an initial concentration of 5 mg g^{-1} soil. The samples were adjusted to the same water potential (90% of field capacity). Duplicate samples were incubated in the dark at 25°C. Samples of sterilised materials (autoclaved three times at 121°C for 15 min) were also included. The containers were opened in a sterile work-station each week, sterile deionised water was added if necessary to maintain the moisture content, the materials were mixed thoroughly and returned for incubation.

2.3 Total viable bacteria

Total viable bacteria were determined in the materials by a standard dilution plating and counting method using tryptic soy agar[13] (3·0 g litre^{-1}) with three replicates. The number of colony-forming units (cfu) was assessed after seven days' incubation at 25°C.

2.4 Atrazine extraction

The dissipation of atrazine was determined by measuring the concentration of atrazine remaining in the samples zero, one and three months after incubation. Triplicate subsamples (10 g) were taken from each container for extraction using acetonitrile + water (9 + 1 by volume). A subsample (10 g) was shaken with aqueous acetonitrile (30 ml) in centrifuge tubes overnight in the dark and then centrifuged for 10 min at 3000*g*. The supernatant was filtered through a special nylon membrane filter (0·2 μm) which was compatible with acetonitrile. Preliminary tests showed that atrazine was not retained by these filters, and 100% recovery was obtained. The filtered extract (1·5–2·0 ml) was collected in glass vials for analysis. The rest of the extracts and the standards used were stored at < 4°C in the dark for further analysis. Samples of extracts and standards were delivered from laboratory to laboratory for verifying the analytical technique. Analytical standards in each country were used as reference samples for the

TABLE 1
Location, Depth (Position of Sampling at the Base of the Core), and Texture of the Samples used in this Study from the Surface (SUR) or Subsurface Unsaturated Zone (SUB)

Sample	*Country*	*Site*	*Depth (m)*	*Texture*
BL-SUR	Belgium	Louvain-la-Neuve	0·15	Loam
BL-SUB	Belgium	Louvain-la-Neuve	11·12	Sand
GR-SUB	Greece	Nafplio	1·97	Clayey silt
HU-SUR	Hungary	Debrecen	0·10	Loam
HU-SUB	Hungary	Debrecen	1·90	Loess
UK-SUR	UK	Reading	0·12	Sandy loam
UK-SUB	UK	Reading	10·24	Sandy clay

comparison of analytical results obtained in each laboratory.

2.5 Atrazine analysis

The concentration of atrazine was measured by HPLC (High Performance Liquid Chromatography) using a UV detector at 220 nm after separation by reverse-phase chromatography on an ODS(30) column (Ultracarb 5; 150 × 4·6 mm) and acetonitrile + water (70 + 30 by volume) as mobile phase. The column was operated at constant room temperature (23(± 1)°C), and the mobile phase flow rate was 1 ml min^{-1}. The sample volume injected was 25 μl. The concentration of atrazine in the extracts in each vial was determined twice under the same conditions.

3 RESULTS AND DISCUSSION

3.1 Total viable bacteria

Table 2 shows that all samples used in this study contained large populations of viable bacteria. These results confirm that bacteria can exist in high numbers in subsurface environments.[14–16] There were differences in data between laboratories for some materials by a factor of up to 10. This variability is not significant ($P > 0{\cdot}05$) and within the range usually encountered in soil materials. Coefficients of variation for log transformed data ranged from 1·2 to 7·4%. Subsurface samples generally contained fewer bacteria than surface samples.

3.2 Atrazine degradation

The protocol used for studying atrazine was developed during various preliminary tests using different methods to determine the extraction efficiency and accuracy of analysis. These preliminary tests showed that the extraction efficiency ranged from 92 to 97% of the total atrazine added into different materials after different times of incubation. These tests also showed that no more atrazine was recovered when the soils were re-extracted with fresh acetonitrile + water.

The assessment of accuracy of the analytical technique for each individual laboratory was made by comparison with the performance and quality control of other laboratories. The analytical results for 15 atrazine samples carried in four laboratories (Fig. 1) shows that the difference among laboratories was not significant ($P > 0{\cdot}05$), indicating that the analytical procedure was reliable.

All laboratories found that there was little or no dissipation of atrazine in sterilised samples (data not shown) indicating that dissipation in non-sterilised samples was essentially biological (i.e. degradation). The results of all laboratories (Figs 2 and 3) indicate the potential for atrazine degradation in the non-sterilised materials used in this study as previously reported for aquifer sediments.[11] Figures 2 and 3 show that there were consistent trends after one month and three months. These data together with those for total viable bacteria (Table 2) indicate that there is no relationship between total viable bacteria and the degradation rate of atrazine in the materials examined here.

The results also show that there was almost complete dissipation of atrazine in surface materials but variable degradation was observed in subsurface samples. Comparison of the results obtained for all materials in all laboratories showed no significant ($P > 0{\cdot}05$) difference among the data obtained in all laboratories. The variation in the results between laboratories was much smaller for surface samples ($P = 0{\cdot}91$ and $P = 0{\cdot}32$ for one and three months' extraction, respectively) than for subsurface samples ($P = 0{\cdot}19$ and $P = 0{\cdot}06$ for one and three months' extraction, respectively). The statistical analysis indicated that variation among laboratories increased as the concentration of detected compound

TABLE 2
Total Viable Bacteria in Surface (SUR) and Subsurface (SUB) Materials as Determined by Laboratories in Belgium (BL-LAB), Greece (GR-LAB), Hungary (HU-LAB) and the UK (UK-LAB)

	Total viable bacteria (*log cfu* g^{-1} *soil*)				
Sample	*BL-LAB*	*GR-LAB*	*HU-LAB*	*UK-LAB*	*CV*[a] (%)
BL-SUR	7·00	6·86	7·06	7·18	1·9
BL-SUB	7·44	6·44	6·57	7·07	6·7
GR-SUB	7·44	7·72	7·57	6·93	4·6
HU-SUR	8·18	6·89	7·18	7·40	7·4
HU-SUB	7·73	6·63	6·84	6·95	6·8
UK-SUR	7·30	7·27	7·28	7·54	1·7
UK-SUB	6·10	5·10	6·83	6·30	1·2

[a] Coefficient of variation.

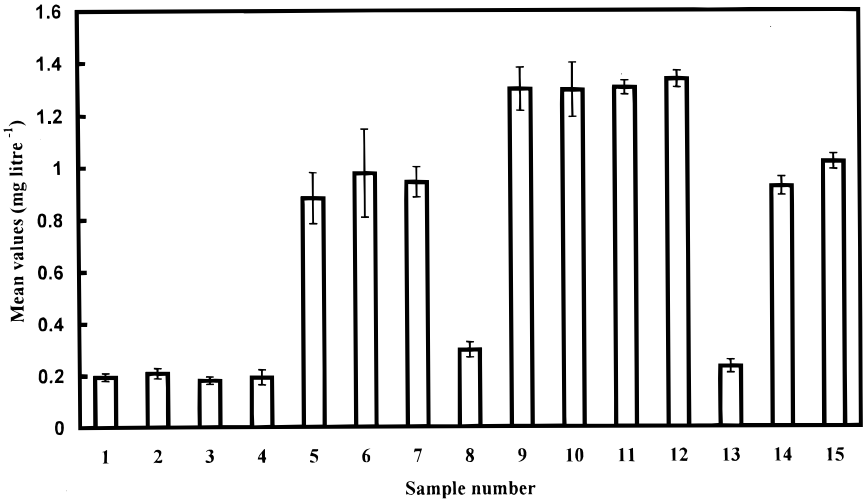

Fig. 1. Analytical results for 15 atrazine samples of different concentrations as determined in four laboratories; error bars are the standard errors based on four laboratory results for each sample.

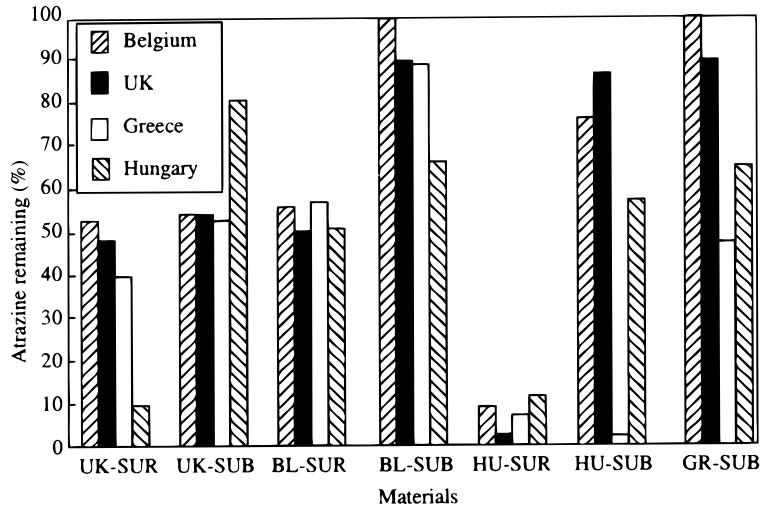

Fig. 2. Dissipation of atrazine in surface (SUR) and subsurface (SUB) materials one month after application as determined in four laboratories (mean of six replicates for each material determined in each laboratory).

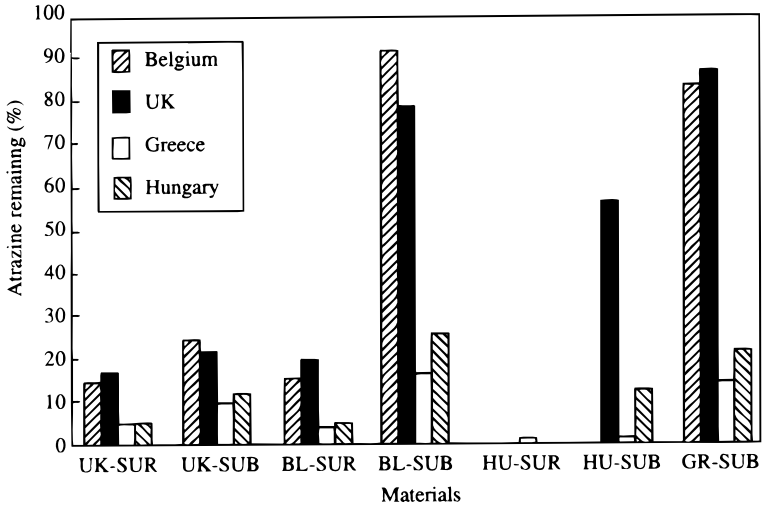

Fig. 3. Dissipation of atrazine in surface (SUR) and subsurface (SUB) materials three months after application as determined in four laboratories (mean of six replicates for each material determined in each laboratory).

decreased. Figures 2 and 3 also show that there were major differences between laboratories for subsurface samples obtained from Belgium and Greece. The statistical analysis for the amount of atrazine remaining in subsurface samples from the UK and Hungary as determined by each laboratory showed no significant ($P > 0{\cdot}05$) variation among laboratories.

The differences between laboratories were not due to (i) differences in experimental protocol, as all laboratories used the same procedures of sample pretreatment, incubation and extraction, (ii) analytical errors as 15 atrazine samples obtained from each laboratory were analysed by HPLC in all laboratories resulting in good agreement (Fig. 1), (iii) differences in total viable bacteria (Table 2) as there was no significant variation among laboratories, and no relationship between total viable bacteria and degradation rate or (iv) physical heterogeneity of samples, as samples were well mixed by one laboratory before dissemination to other laboratories.

We suggest that heterogeneity of materials in terms of, for instance, the distribution of specific micro-organisms present in relatively low numbers is most likely to have caused the variation in degradation rate between laboratories in some samples. Homogenisation at the macro-scale does not necessarily mean that the atrazine-degrading micro-organisms were equally distributed among subsamples of subsurface materials at the micro-scale. For example, the subsamples of materials (BL-SUB and GR-SUB) used by BL-LAB and UK-LAB probably contained very few atrazine-degrading micro-organisms resulting in very low degradation capacity compared to subsamples of the same materials used in GR-LAB and HU-LAB. This might be avoided by increasing the size of subsample used.

4 CONCLUSIONS

In general, it seems that the variability in data obtained for total viable bacteria is within the range usually encountered in soil material. There was good agreement between laboratories for data on atrazine degradation in surface and some subsurface samples. The differences between laboratories for some subsurface samples were suggested to be at least partly due to a small population of atrazine-degrading micro-organisms which were not equally distributed in the subsamples distributed to the different laboratories. Overall, these data are reassuring and allow further data from all laboratories to be interpreted with confidence.

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